DEFENCE R&D DÉFENSE

Biological Detector Performance with a 402 nm Laser Diode

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Technical Report

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Abstract

A previous generation of biological detection instruments, pioneered at DRES, used light sources in the 340-360 nm region. Although this wavelength region has been shown to excite important biomolecules related to live organisms, implementing them turned out to be extremely expensive due to complex technologies involved. An alternative light solution became imperative. In this paper, biological aerosol measurement was done with a laser diode emitting at 402 nm. It was chosen for its ability to excite a different class of biomolecules. There is a need to demonstrate that a fluorescence aerodynamic particle sizer (FLAPS) perform satisfactorily using these selected types of light sources when presented with a range of biological aerosol simulants. The findings indicated comparable fluorescence response from the select excitation sources in the presence of spore aerosol. Spores, by their physiological nature, contain very little biological material and the individual units are no larger than 0.7 μm and enclosed by a very refractile spore coat. Due to this refractile characteristic, conventional light microscopy of spores reveal very little of their cellular content. In contrast, vegetative cells are better subjects for examination in that they can easily be stained. Thus, performance of biological detectors must be rated by their ability to measure spore aerosols and in these experiments, it has been shown that 10 Agent Containing Particles Per Liter of Air (ACPLA) could be detected.

In summary, we have shown that by using a small laser diode that emits at 402 nm, it is possible to replicate the performance of 340-60 nm light sources. The findings from this study have critical implications for the future of biological detection, both for military applications as well as in environmental monitoring. With this new light source, a detector will require no active cooling system, consume little power and weigh much less than current instruments. The associated cost reduction will not only benefit defence applications but will make it affordable for industrial or home use.

Résumé

La génération antérieure des instruments de détection biologique, innovée au CRDS utilisaient des sources lumineuses aux alentours de 340 à 360 nm. On a déjà montré que cette région de longueur d'onde produit l'excitation de biomolécules importantes reliées aux organismes vivants mais il se trouve que son implémentation demeure extrêmement coûteuse à cause de la complexité des technologies qui y sont rattachées. Il est ensuite devenu impératif de trouver une alternative à cette luminosité. Cet article fait état des mesures biologiques d'aérosols qui ont été effectuées avec une diode laser émettant à 402 nm choisie à cause de sa capacité à exciter des catégories différentes de biomolécules. Il s'agit maintenant de prouver que les séparateurs granulométriques aérodynamiques à fluorescence (FLAPS) fonctionnent de façon satisfaisante au moyen des types sélectionnés de sources lumineuses auxquelles est exposée une gamme de stimulants biologiques en aérosol. Les résultats indiquent que la réaction en fluorescence provenant des sources d'excitation sélectionnées en présence des spores en aérosol était comparable. Les spores, de par leur nature physiologique, contiennent très peu de biomatériaux ; les unités individuelles ne dépassent pas 0.7 μm et elles sont enveloppées d'une couche de spores qui est très réfrangible. À cause de cette caractéristique de réfrangibilité, la microscopie photonique conventionnelle des spores révèle très peu leur contenu cellulaire. Par contre, les cellules végétatives sont des meilleurs sujets à examen parce qu'elles peuvent facilement être contaminées. Par conséquent, le rendement des détecteurs biologiques doit être évalué selon leur capacité à mesurer les spores en aérosol et ces tests prouvent qu'il est possible de détecter 10 particules contenant un agent par litre d'air (ACPLA).

En résumé, nous avons prouvé qu'en utilisant des petites diodes laser qui émettent à 402 nm, il est possible de répliquer le rendement des sources lumineuses de 340 à 360 nm. Cette étude aboutit à des conclusions ayant des implications décisives pour l'avenir de la détection biologique, pour les applications militaires aussi bien que pour la surveillance automatique de l'environnement. Grâce à cette source lumineuse, le détecteur n'exigera pas de système de refroidissement, consommera peu d'énergie et pèsera beaucoup moins que les instruments actuels. La réduction des frais connexes ne concernera pas seulement aux applications du domaine de la défense mais rendra cette technologie abordable pour l'industrie et l'utilisation à domicile.

Executive summary

In this paper, we report on an improvement to the fluorescence aerodynamic particle sizer (FLAPS) technology. DRES invented the FLAPS as the biodetection front end that runs 24 hours by 7 days (24x7) without costly replenishment requirements. As of 2001, it is still the only reliable biological detector design. The frequency tripled laser light source in FLAPS2 has been replaced by a much smaller laser diode, with the potential to reduce cost by several orders of magnitude. The main focus of this paper will be to demonstrate that the instrument with this new light source performs similarly to previous generations of FLAPS.

Laboratory and field trial data indicate that FLAPS performance using the two light sources gave very similar performance characteristics when presented with a variety of different biological aerosol simulants. Most encouraging was the similar fluorescence response from both excitation sources in the presence of spore aerosol, a material considered the most difficult to detect optically. Spores, by their physiological nature, contain very little biological material and the individual units are no larger than 0.7 μm and enclosed by a very refractile spore coat. Due to this refractile characteristic, conventional light microscopy of spores reveal very little of its cellular content. In contrast, vegetative cells are better subjects for examination in that they can easily be stained. Thus, performance of biological detectors must be rated by their ability to measure spore aerosols and in these experiments, it has been shown that 10 agent containing particles per liter of air (ACPLA) could be detected.

The modified FLAPS uses a CW Nichia laser diode emitting at 402 nm instead of the CW HeCd laser originally used. This shift in wavelength could also change the excited molecular species from NADH to riboflavin (Selbach and Kuhlmann, 1999, Van Schaik et al 1999 and Solovieva et al 1999).

In summary, we have shown that by using a small laser diode that emits at 402 nm, it is possible to replicate the performance of 340-60 nm light sources. The findings from this study have critical implications for the future of biological detection, both for military applications as well as in environmental monitoring. With this new light source, a detector will require no active cooling system, consume little power and weigh much less than current instruments. The associated cost reduction will not only benefit defence applications but will make it possible for industrial or home use.

Sommaire

Cet article fait état d'une amélioration de la technologie du séparateur granulométrique aérodynamique à fluorescence (FLAPS). Le CRDS a inventé le FLAPS qui est au premier plan de la biodétection fonctionnant 24 heures par jour, 7 jours par semaine sans exiger de réapprovisionnement coûteux. Depuis 2001, il est toujours le seul concept de détecteur biologique qui soit fiable. La source lumineuse laser dont la fréquence avait été triplée dans le FLAPS2 a été remplacée par une plus petite diode laser ayant le potentiel de réduire amplement les coûts. Le but principal de cet article est d'établir la preuve que cet instrument utilisant cette nouvelle source lumineuse a un rendement égal à celui de la génération antérieure des FLAPS.

Les données de laboratoire et les essais en champ indiquent que le rendement des FLAPS qui utilisent les deux sortes de sources lumineuses atteignent des caractéristiques de rendement très similaires quand ils sont exposés à une variété de différents stimulants biologiques en aérosols. L'aspect le plus encourageant est que ces deux sources d'excitation ont provoqué une réaction en fluorescence similaire lorsqu'elles ont été exposées aux spores en aérosol, un matériau que l'on considère comme le plus difficile à détecter optiquement. Du fait de leur nature physiologique, les spores contiennent très peu de biomatériau et les unités individuelles ne dépassent pas 0.7μm et sont enveloppées d'une couche de spores qui est très réfrangible. À cause de cette caractéristique de réfrangibilité, la microscopie photonique conventionnelle des spores révèle très peu leur contenu cellulaire. Par contre, les cellules végétatives sont des meilleurs sujets à examen parce qu'elles peuvent facilement être contaminées. Par conséquent, le rendement des détecteurs biologiques doit être évalué selon leur capacité à mesurer les spores en aérosol et durant ces tests, on a montré qu'il est possible de détecter 10 particules contenant un agent par litre d'air (ACPLA).

Le FLAPS modifié utilise une diode laser à onde entretenue Nichia émettant à 402 nm au lieu du laser HeCd à onde entretenue qui avait été utilisé à l'origine. Ce décalage de longueur d'onde pourrait aussi transformer les espèces moléculaires excitées du NADH à la riboflavine (Selbach and Kuhlmann, 1999, Van Schaik et al 1999 et Solovieva et al 1999).

En résumé, nous avons prouvé qu'en utilisant des petites diodes laser qui émettent à 402 nm, il est possible de répliquer le rendement des sources lumineuses de 340 à 360 nm. Cette étude aboutit à des conclusions ayant des implications décisives pour l'avenir de la détection biologique, pour les applications militaires aussi bien que pour la surveillance automatique de l'environnement. Grâce à cette nouvelle source lumineuse, le détecteur n'exigera pas de système de refroidissement, consommera peu d'énergie et pèsera beaucoup moins que les instruments actuels. La réduction des frais connexes ne concernera pas seulement les applications du domaine de la défense mais rendra cette technologie abordable pour l'industrie et l'utilisation à domicile.

Table of contents

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INTRODUCTION

Producing an optical instrument that unequivocally measures biological content has been difficult. To perform this task, we have reported on the first generation fluorescence aerodynamic particle sizer (FLAPS1); we demonstrated single biological spore particles in an air stream could be detected (Hairston et al. 1997). It was effective in field trial detection of biological aerosol where no false alarms were encountered (Boulet et al. 1996). Background biological aerosol characteristics measurement using the FLAPS has been described (Ho and Spence, 1998). More recently we described the performance characteristics of FLAPS2, a field portable instrument optimized for rapid biological aerosol detection and environmental monitoring. It was shown that fluorescence measurements in field trials could be correlated with viable particles (reference data). Reference data sets were provided by an independent source and expressed in live or culturable biological particles per unit volume of aerosol (Ho et al. 1999).

The excitation light source in FLAPS2 is a frequency tripled device that is not only expensive, it is also sensitive to environmental influence. For example, it has a narrow temperature range requiring an active cooling system for optimal operation. Shock mounting is also needed to ensure all the optical elements are in alignment. As a result, this light source severely restricts design flexibility. Clearly, a robust light source will make FLAPS a more versatile instrument.

Apart from military applications, FLAPS can be useful in environmental biological measurements. There has been much public concern for air quality, related to the known sources of pollution from chemical and biological origins and their perceived health effects. Chemical monitoring and concentration standards have been instituted in many communities. Progress for similar directions in biological monitoring has been lacking, due to a number of technical problems. There have been no economical methods for continuous measurement of biological aerosols.

Moreover, biological aerosols, mostly harmless, are indigenous to many environments. Microorganisms are naturally aerosolised in the atmosphere, often becoming a biological burden to downwind communities. For example, municipal wastewater aeration tanks have been studied and found to produce, by the minute, over 130 viable particles per m^3, about 10% being coliforms (Sawyer et al. 1993). Hosing down of dairy processing plants was shown to produce a biological aerosol that remained above ambient concentrations for as long as 40 minutes (Kang and Frank, 1990). Diffusion modeling showed that aerosolised microorganisms could spread infection over a 150 km^2 area among 10 swine herds (Grant et al., 1994). Indoor microbiological air quality in homes and offices has been reported for bacteria and fungi (Lis et al. 1997). The bacterial numbers ranged from 200-800 (homes) and 100-500 (offices) viable particles/m^3 while those for fungi were about half as abundant. Fairly similar findings were reported by the US Environment Protection Agency which has recently taken an interest in measuring indoor and outdoor biological aerosol quality (EPA, 1998).

Before meaningful health assessments can be made, it is clear that extensive biological aerosol measurements have to be carried out over long periods of time. The job can be executed more efficiently if it can be done in real time without having to resort to using conventional but resource intensive microbiological growth assessment methods. One possible solution is to adopt a two step approach. Step one uses an optical technique that can be run 24 hours a day, 7 days a week without requiring liquid reagents or costly expendable material. Step two is triggered when the optical instrument detects a potential biological aerosol of significant concentration. Samples are collected and conventional microbiological assessment and clinical chemistry can be performed to identify the biological agent. The FLAPS instrument can serve as the optical front end if it can be made affordable.

In this paper, we report on our continuing attempts to improve the FLAPS technology. The frequency tripled laser light source in FLAPS2 has been replaced by a small laser diode, with the potential to reduce cost by several orders of magnitude. However, the laser diode produces 402 nm wavelength light, quite different from the original device at 350-352 nm. The immediate concern is that the longer wavelength may not provide measurement characteristics comparable to the reference standard. The main focus of this paper will be to demonstrate that the instrument equipped with a cheaper light source produces data comparable to previous generations of FLAPS instruments.

MATERIAL AND METHODS

Solid State Light Source

From the factory, a high output pre-production laser diode capable of 15 mW output was obtained. The technical literature also called this device a "Violet Laser Diode", type NLHV500A, 402 nm (Nichia Corporation, 491 Oka Kaminaka-Cho Anan, Tokushima 774-8601, Japan). The laser was driven by a model LDD-200-2M Laser Diode Driver, from Wavelength Electronics, Bozeman, MT.

The violet laser diode was operated in constant current mode, with a power readout connected to the laser's internal photodiode. The power droops as the laser warmed over the course of each trial, so during initial startups manual adjustments were made to maintain laser output power between 12 and 14 mW. At times, the power droop and compensating manual adjustments may affect data displays as slight distortions in the fluorescence intensity data.

Output beam was collimated by a precision lens (Model 307-4606-670, 4.6 mm focal length, numerical aperture 0.53, Optima Precision, Inc., West Linn, OR).

To prevent the excitation light from reaching the fluorescence detector, a long pass filter with cut off wavelength at 435 nm was used (Schott Glass GG 435, Melles Griot, Irvine, CA). The filter is used in addition to other existing filters for the FLAPS1 (Hairston et al. 1997), and installed only when the Nichia laser is operated. When in use, the GG435 filter is located between the primary light collection lens and the dichroic filter.

To facilitate a convenient choice of light source selection a flat mirror (02 MFG 015/038 Melles Griot, Irvine, CA) on a Kinematic mirror mount (BKL-4, Newport Corporation, Irvine, CA) could be inserted along the exciting beam path. This steering mirror for the 402 nm laser beam was installed in the path of the 325 nm HeCd beam. The Kinematic base was removed for 325 nm operation without requiring optical realignment.

Fluorescent Aerodynamic Particle Sizer (FLAPS).

With DRES specifications, TSI (St. Paul, MN 555164-0394) built the FLAPS1 using a HeCd CW laser source coupled to their existing aerodynamic particle sizer (APS). The success of this project led to a second contract to construct FLAPS2 based on their APS II. This instrument, FLAPS2, is now designated as model 3312a; for marketing (TSI) reasons it's also called "Ultraviolet Aerodynamic Particle Sizer" (UV-APS). The label "FLAPS" is used in this report to describe the instrument that, with custom software performs data analysis with advance display and alarming capabilities. In contrast UV-APS is marketed with minimal analysis software.

Proprietary display and alarming software was developed by DRES with Dycor (Edmonton, Alberta) as programming contractor. The original APS particle analysis code, in development since mid 80's, was used in DRES field biological detection. It evolved into a functional tool, used successfully during international field trials (Ho et al. 1995). Further development work brought the software up to date and

in its present form is used to control the FLAPS. The name "FLAPS" describes a functional package consisting of the fluorescence enabled APS hardware and DRES software combination.

The FLAPS2 configuration was equipped with a pulsed UV light source ("StableLight" model S349-30Q, Uniphase, 163 Baypointe Parkway, San Jose, CA 95134, 800-644-8674). It was powered by a diode-pumped Nd:YLF laser frequency tripled to produce 349 nm. Energy per pulse was rated at 3 mJ with a pulse width of about 5 to 8 nsec. Pulse power was regulated by software to provide an optimal level. This resulted in lower background noise and better signal-to-noise characteristics. The UV laser was triggered by the time-of-flight signal when a particle was detected.

During real time sampling, the instrument presents particle size and fluorescence intensity information for each particle. Data derived from a sampling period, typically 3 seconds, can be reduced to a fractional number (gated % fluorescent); this represents the percent of particles that exhibited fluorescent signal above a preset size and intensity threshold. A threshold level can be determined for each environmental condition by observing the prevailing background conditions. An alternate method to present fluorescence data is to derive a "signal-to-noise" ratio. This is done but comparing the mean of background gated % fluorescent with new "unknown" or "biological" data.

To increase the population of background fluorescent particles for better statistical counting, a prototype aerosol concentrator (model XMX, SCP Engineering, St. Paul, MN) was used as a front end to the FLAPS2 intake. This was a modified version of the original XM2 used for concentrating biological aerosol, as reported previously (Brenner et al. 1988). The present work employed a smaller version, designed for optimal size, weight and power consumption. It concentrated 400 liter/min into a 1 liter/min flow to the FLAPS2 intake. Improved particle throughput provided by this setup facilitated a rapid sampling time of 3 seconds. In practice, an additional 1 second penalty was incurred for computational and data handling overhead. By this protocol, aerosol data could be collected continuously every 4 seconds over period of days unattended by the operator.

Fluorescent Bead Aerosol

When working with the 402 nm light source, 2 μm fluorescent latex beads were used (catalog number B0200, Duke Scientific Corporation, Palo Alto, CA). The blue fluorescent dye has 3 excitation peaks: 365 nm, 388 nm, and 412 nm. For 365 and 388 nm excitation the main emission peak is at 445 nm. For 412 nm excitation, the 445 emission peak remains and there is a secondary emission peak at 473 nm. In our application, 402 nm falls on the sides of both the 388 and 412 excitation peaks, resulting in a combination from the 445 and 473 nm emission peaks.

Beads were aerosolized using a TSI Model 3076 nebulizer, together with a TSI 3074B air supply filter and a TSI 3012 aerosol neutralizer.

Aerosol Chamber

Biological aerosol dissemination (spores of *Bacillus subtilis* var *niger*, BG) was accomplished with a Hudson nebulizer (model 1700, Hudson Oxygen Therapy Sales Co.,Wadsworth, OH) driven by a 172-206 KiloPascal (25-30 psi) air line. A suspension containing BG in the range of 5 to 30 μg plus 4 mg silica gel (Syloid 245, Davison Chemical, Baltimore, MD) per ml was used as the starting material. For each experiment, the dry BG powder was weighed out from a stock batch. For example, to prepare a 10 μg/ml suspension, 0.1 gm dry powder was weighed out (model AE 200-S, Mettler Instruments, Zurich, Switzerland) and added to 10 ml distilled water. Serial 1:10 dilutions were repeated 3 times to obtain a final concentration of 10 μg/ml. A working volume of 25 ml was used to fill the aerosol generator. Actual viable counts in the suspension were determined to obtain the source concentration.

A steady aerosol concentration in a 90.5 m^3 chamber was achieved via a feed back control loop mechanism as previously described (Ho, 1989). In this scheme, a particle sizer was used to measure aerosol concentration at every 5 second interval. The result was compared to a previously defined threshold; custom software performed the control function to regulate switching of the aerosol generator. The monitored particle size range was 0.7-1.0 μm at 1 particle per cc concentration threshold. Steady state aerosol concentration was achieved in about 15 minutes from startup. Syloid was used to provide sufficient particle counts in the aerosol to maintain efficient operation of this feedback loop. Using μg/ml quantities of BG without presence of the Syloid would not generate sufficient particles to perform the feed back looping operation.

For larger volumetric sprays, the Ulvafan Mk 2 (Micron Sprayers Ltd, Bromyard Industrial Estate, Bromyard, Herefordshire, HR7 4HS, UK) was used. This is an air-assisted spinning disc sprayer designed for low and ultra-low volume application of spray liquids in situations with no natural air movement, like in the chamber. The sprayer produces small even sized droplets that are carried in an air stream, resulting in aerosol particles on drying. A 12 volt D.C. portable rechargeable battery powers the instrument. A 100 ml slurry containing 0.1% W/V BG was used as spray material.

Field Trial Protocols.

Field biological aerosol dissemination (spores of *Bacillus subtilis* var niger, BG) and reference sample collection were done at DRES. Biological aerosol dissemination took place about 100-250 m upwind of the detection system as detailed in the procedure by Ho and Spence (1996). The procedure used at DRES is similar to that used at Dugway Proving Ground. Andersen and Mohr (1996) have described the procedures in detail and only brief comments will be stated here where warranted.

To obtain reference data, culturable particles in air were captured and grown to determine the presence of "live" content. Biological aerosol particles were impacted onto a 15 cm nutrient agar plate mounted in a slit sampler (model STA 203, New Brunswick Scientific, Edison, NJ 08818-4005). The collection flow rate was 50 liter/min. Using custom circuits, each sampler was electronically programmed to rotate at 1 revolution in 2 min. A sampler array consisted of 10 devices serially connected to function as a continuous 20 minute collector. The sampling station was located on an elevated platform, set at 1 m from the FLAPS under field test.

On passage of a test cloud, the reference samplers were switched on manually by the trial operators. Switch timing was estimated from the moment of cloud arrival as indicated particle number measurement (APS). In practice, the slit sampler array were turned on about 30 seconds while still measuring background aerosol. Precise timing was critical for effective operation to provide useful reference data bracketed with background aerosol information before and after passage of a cloud. During sampling of a cloud, biological particles, if present in the aerosol, were impacted on to the surface of a nutrient agar plate. After over night incubation, live particles were counted as bacterial colonies by means of a flat bed scanner driven by custom software developed jointly by DRES, DPG and Spiral Biotech (model "STAR" version 1.5, Spiral Biotech, Bethesda, MD 20814; due to merger of this company with another, this instrument is no longer commercially available). Reference data were expressed as agent containing particles per liter of air (ACPLA). In this report, fluorescent data were compared to reference data to illustrate timing relationships whenever biological hits occurred.

In experiments that required a protein aerosol simulant, egg albumin powder (A5253) was used. It was obtained from Sigma Chemical Co and particulate aerosol was produced by a Nordson Tribomatic® 500 spray gun (Canwest Pumping Systems Ltd Calgary, Alberta T2H 0S9 Canada) as a dry aerosol.

Statistical Analysis and Data Transform.

To test if two variables are interdependent or vary together, the common approach is to perform a correlation analysis (Sokal and Rohlf, 1981). It is assumed that the two variables are both effects of a common cause. Translated to our present problem, we wish to estimate the degree to which 2 FLAPS instruments measured the same live biological aerosol. Before applying this test, there is one precaution to note- the data must be tested for distribution normality. Non-normally distributed data like FLAPS measurements and slit sampler data (shown in results) require non-parametric methods. Spearman's non-parametric correlation coefficient was determined by SigmaStat version 2 (SPSS Inc. 444 N. Michigan Avenue, Chicago, Illinois, 60611) as described by Sokal and Rohlf (1981). This test requires 2 data sets of equal size and timing intervals. However, FLAPS data sets derived from two different instruments, have different timing entries. As a consequence, they are structurally dissimilar and unsuitable for this type of analysis.

Ho et al (1999) have described the method for obtaining data sets with equal size and timing intervals. Each data set consisting of time and the variable was transformed by first converting the time format into decimal time (e.g. 12:00:00 to 0.5) using the "timevalue" function in Excel (Microsoft Office 97, Microsoft Inc., Redmond, Washington 98052-6399 U.S.A). Then x and y data columns were imported into TableCurve 2D (version 4, SPSS Inc. 444 N. Michigan Avenue, Chicago, Illinois, 60611) where non-parametric "local regression" estimation was done using Gaussian weighting. For generating new interpolated data sets, increment decimal time value of 0.000023148 or about 5 sec. was used along with specified beginning and end times. After performing the conversion procedure for both FLAPS data sets, just the y portions of each set were grouped, constituting the variable pair suitable for Spearman's test.

RESULTS AND DISCUSSIONS

Analysis of Fluorescent Standard Latex Beads

As a calibration check for good optical alignment, standard fluorescent latex beads were examined with FLAPS1 using the Nichia light source. Shown in figure 1 is the plot of fluorescence brightness as a function of intensity channel versus number population. The data conformed well to a Gaussian distribution, with a fit correlation coefficient of 0.96. The results suggest that the optical components were properly installed and optimized for fluorescent detection of particulate aerosols.

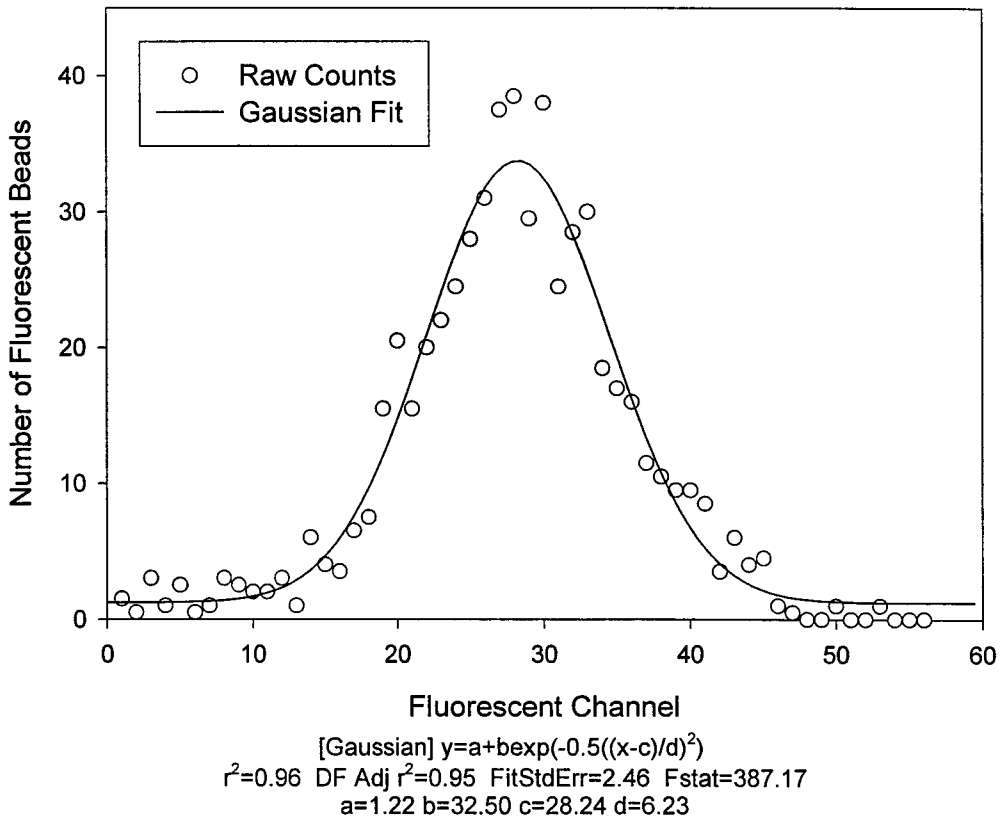

Figure 1. Analysis of standard fluorescent latex beads. Measurements were obtain with the FLAPS1 platform using aerosolized 2 μm fluorescent latex beads. Excitation was by the Nichia laser diode at 2.75 mW with detector PMT voltage set for 750V. Very tight size distribution was observed for the 2 μm beads, with all the counts appearing in 2 channels. The raw number counts were an average of the 2 size distribution channels. Curve fitting was done with the raw data set and yielded a Gaussian distribution r^2 fit (0.96).

Measurement of Biological Aerosol in Chamber

The objective of this demonstration (figure 2), was to measure the background particles in a chamber followed by gradual introduction of a spore aerosol. It was anticipated that if the standard 1 liter/min flow was attenuated (to 0.64), there might be a better signal to noise measurement due to slower particle transit time in the laser diode beam. Thus the experiment was started with the slow sample flow rate. As shown in the figure, at the start of the sequence, there was no difference in fluorescence signals after turning on the light source. This suggests that the HEPA filtered air in the chamber contained few, if any background material that had fluorescence characteristics. Shortly after the aerosol spray was introduced, particles with fluorescence signals could be detected at around 14:41:00 or thereafter.

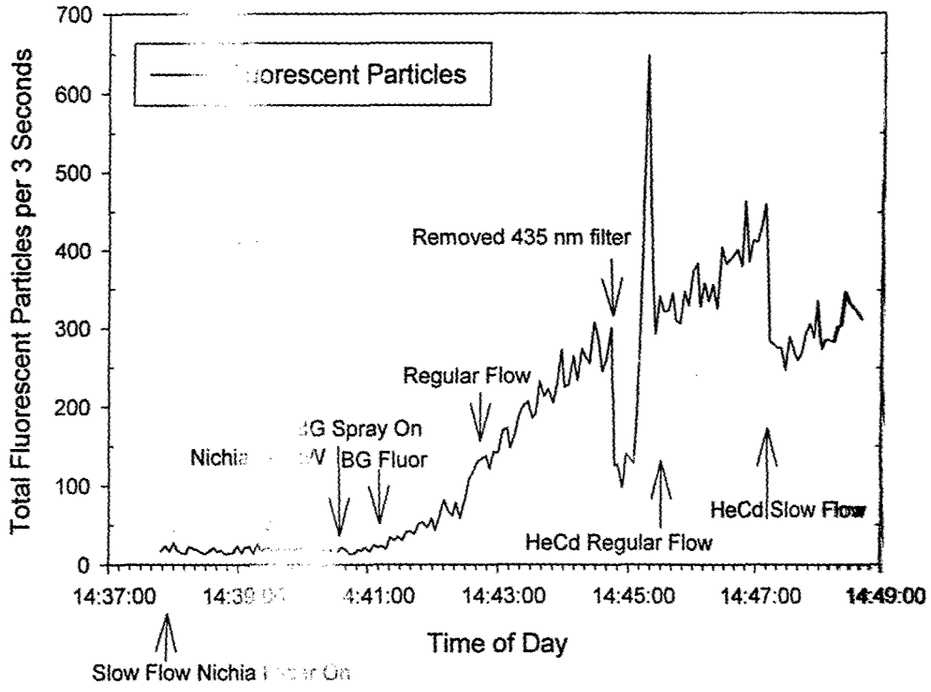

Figure 2. Characteristics of BG aerosol from a 0.1% slurry generated with a spinning disk sprayer. FLAPS1 was operated at normal aspiration without concentrator. At the beginning of the run, the 1 liter APS flow rate was slowed down to enhance capturing weak fluorescent signals. At 14:39:25, the Nichia laser voltage was set at 14 mW; at 14:40:32. BG spray was turned on; at 14:41:13 first visible BG fluorescent signals appeared. at 14:42:?? regular 1 liter flow rate was reset; at 14:44:40 removal of the 435 nm filter was executed in preparation for switching light source; at 14:45:?? the HeCd laser was used at regular flow rate and finally at 14:47:09 still using the HeCd, the slow flow rate was cut in.

As the build up of particles progressed, sample flow rate was restored to 1 liter per minute just before 14:43:00. This appeared to have some but slight effect on the rate of detection as observed from the slope of the trace. The measurement rate showed a steady state progress up to 14:44:30 when a switch in light source took place. This action can be seen as a dip in the data trace at about 14:45:00. Converting from the Nichia laser to the HeCd laser involved removal of the 435 nm blocking filter, removal of the diverting mirror and restoring the sample flow (illustrated by the large signal spike). It is remarkable to note that after switching to the HeCd source, the data trace resumed a similar rate of measurement as the previous conditions (at 14:46:00). Moreover, when the flow rate was attenuated to the same level as the beginning of the experiment, the fluorescent particle sampling rate dropped significantly, at least by visual inspection. This lower rate can be seen to be similar to that measured at the start of the test.

The implication for this observation is that even though the two light sources were swapped in and out during a steady state increase of BG particles in the chamber, the measurements under the two conditions yielded very comparable results. A different view of this conclusion can be expressed by examining the particle size distribution (Y axis) versus fluorescence brightness (X axis) versus concentration plot (Z axis) as shown in figure 3. This is the same experiment as in figure 2 but showing snap shots of the BG particle samples as an individual 3 second time slice at different points of measurement progression. Panel 3a shows clean air containing minimal fluorescent particles while the next panel depicts the beginning of spore detection. In panel 3b, the apparent large particles do not reflect the actual size distribution due to the reduced flow rate that has an affect on the size calibration. Recall that in the APS, particle size is a function of the time-of-flight measurement calibrated to 1 liter/min flow rate. In panel 3c where regular flow rate was restored, the plot of size distribution is the true representation of the particles being measured. After switching to the HeCd light source, it can be seen that in panel 3e, the measured size and brightness characteristics were similar to that for the Nichia source (panel 3d). In contrast, when the flow rate was reduced as shown in panel 3f, there was a significant shift in the resultant size distribution towards larger particle size. This is consistent with the fact that larger particles travel slower than small ones.

In another BG aerosol chamber test, we attempted to correlate fluorescent particle measurement with live particles that form colonies on nutrient agar plates. The results can be seen in figure 4 where at the start of the aerosol spray, fluorescent particles could be shown to increase steadily over time. At 10:00:00, slit sampling was started to collect for 4 minutes. The results of the live particles were expressed as agent containing particles per liter of air (ACPLA). The aerosol generator was a spinning disk device designed to produce relatively high concentration of particles >2μm and this can be seen by the 200 to 400 ACPLA values shown in figure 4. To capture live particles in high resolution with respect to time, the samplers were run to resolve particulate material in 1 second time slices over a 4 minute time span. It can be seen that in figure 4, the rate of increase of fluorescence roughly paralleled that of the live particles. In figure 5, the same reference data is shown in expanded time scale

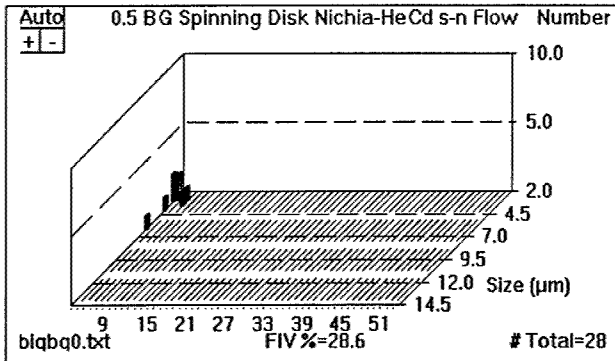

Figure 3a. Background with Nichia laser set at 14 mW

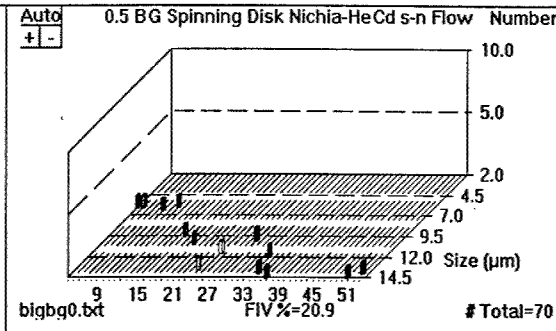

Figure 3b. After sprayer was turned on, first appearance of fluorescent signals

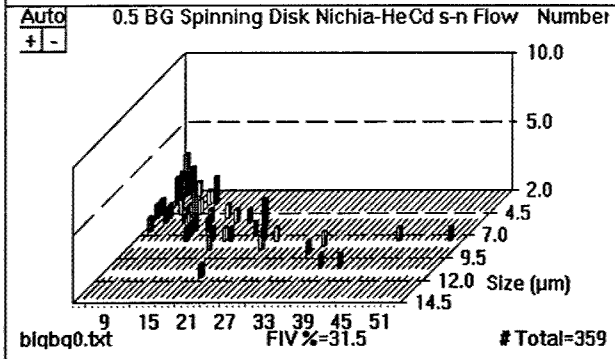

Figure 3c. Regular 1 liter flow rate was established

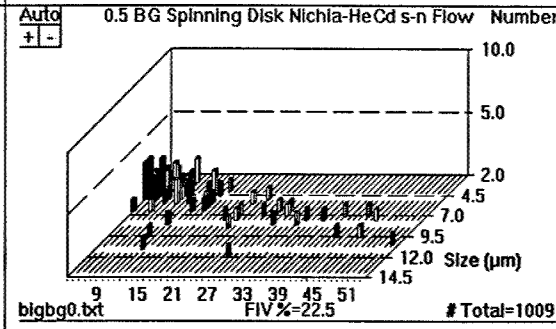

Figure 3d. Before removal of 435 nm blocking filter, still using the Nichia laser

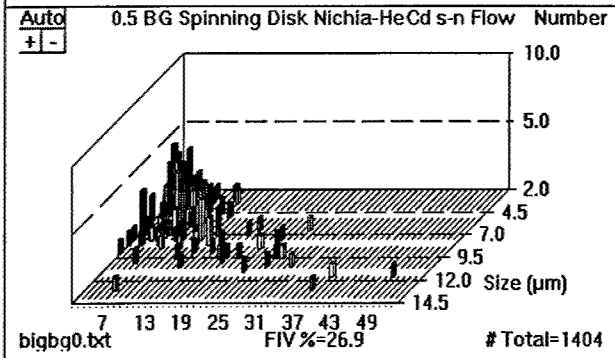

Figure 3e. After removal of the 435 nm filter and switching over to the HeCd laser at 12 mw

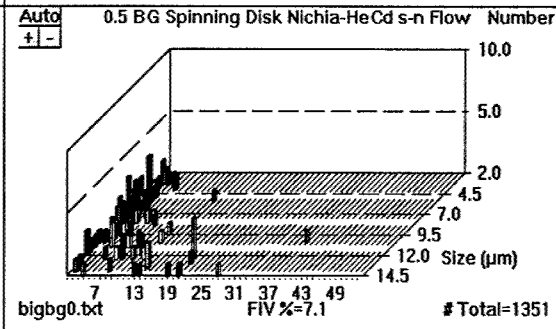

Figure 3f. Slow flow was reintroduced resulting in fewer particles counted

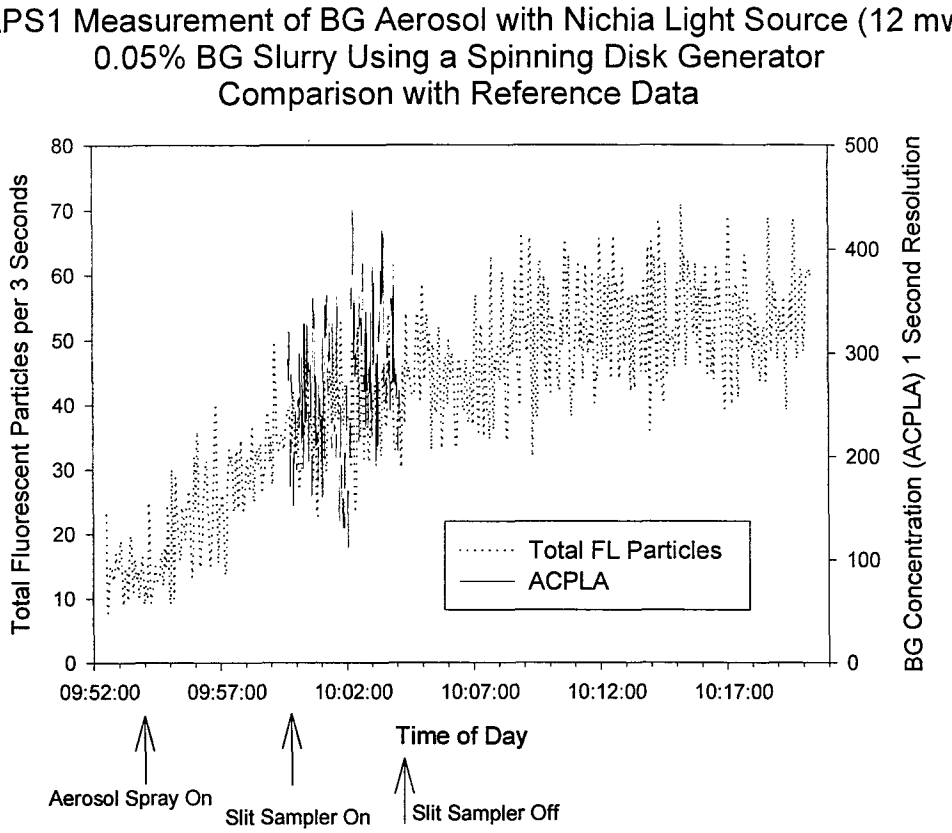

Figure 4. Measurement of BG spore aerosol in a chamber. Comparison of optical data versus reference data

of 4 minutes giving the opportunity to inspect and compare the dynamics of the two measurement methodologies. The arrows in the figure provide subjective indications as to when the two data sets appear to coincide.

It is not possible to state unequivocally that in figure 5 we have data to claim that the two measurement systems were tracking exactly the same aerosol concentration fluctuations. We know that the inlet nozzle for the FLAPS instrument was located outside the chamber attached to a wall mounted sampling port via a 2 meter length of hose. Also, the slit samplers were located inside the chamber but about 2 meters from the wall where the external sampling port resided. Thus spatially there was a significant distance for the aerosol concentrations to establish variations.

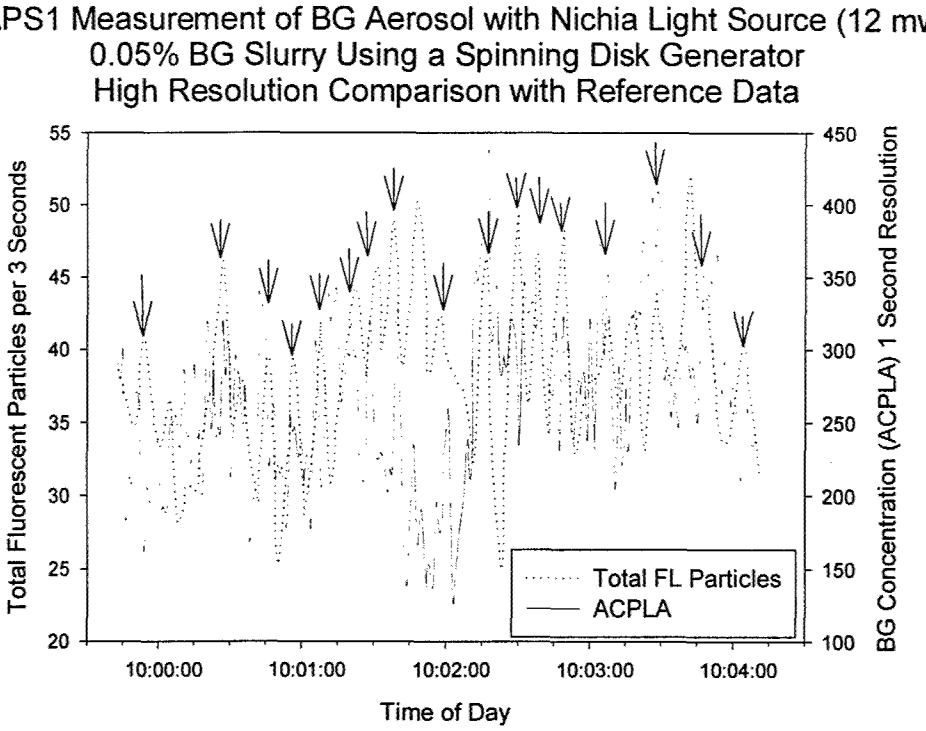

Figure 5. High resolution examination of optical signals and reference data. The data segment was taken from figure 4 with an expanded time axis. Arrows show subjectively where the two measurements appeared to coincide.

On the other hand, there were fans within the chamber that assisted in even mixing so that it was reasonable to expect aerosol concentration uniformity at least up to the wall mounted sampling port. Finally, there was a transit time for the particulate material between the port inlet to the FLAPS optical sensors and this could cause the bulk of the errors. Given this caveat, it is remarkable to note the large number of coincident measurement episodes denoted by the arrows. A simple observation from this experiment is that the optical measurement system appeared to be capable of tracking the dynamics of a live biological aerosol in real time with fairly good fidelity.

Comparison of Three Light Sources

A FLAPS2 instrument powered by a frequency tripled YAG light source was used to measure BG aerosol simultaneously as the FLAPS1 platform that had two interchangeable light sources. Similar experimental approach was used to supply a dynamic BG aerosol concentration in the chamber while optical measurements were recorded. Shown in figure 6 are the results of this comparative test. At the start of the test, the Nichia laser was selected in FLAPS1, running at the slow flow rate. A short time after the aerosol spray started, both detectors measured the presence of

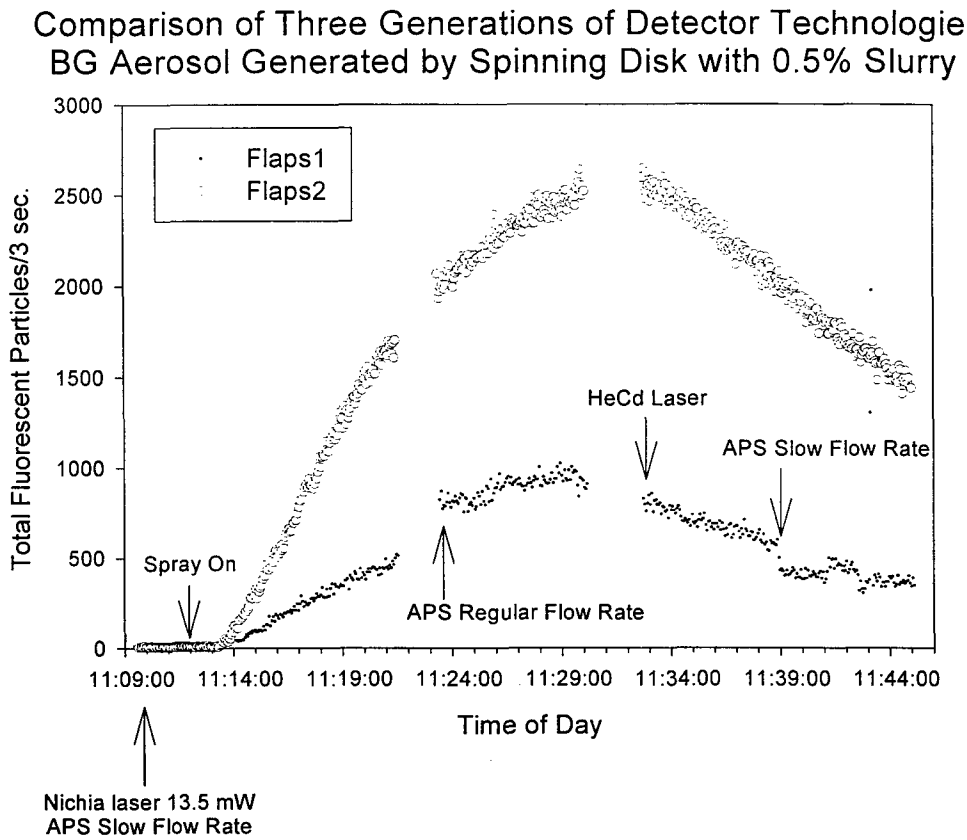

Figure 6. Excitation of biological aerosol using a laser diode (Nichia), gas laser (HeCd) and a frequency tripled solid state YAG (Flaps2). The two instruments were connected to sampling ports of an aerosol chamber.

fluorescent particles. It can be seen that their absolute counts were dissimilar, the pulse laser instrument (FLAPS2) registered higher measurement rate. Perhaps a reasonable explanation can be as follows. The light source in FLAPS2 was a pulsed device thus was expected to provide a more energetic excitation for each particle. Consequently, small particles not normally excited by a less powerful beam (CW) will provide a measurable emission signal. This small particle population added to the larger ones, resulted in more particles fluorescing per unit time. No attempts were made to detune the FLAPS 2 light source.

At about 11:24:00 when FLAPS1 was switched to run on normal sampling flow rate, it can be seen that the two instruments registered roughly similar particle sampling rates. When the spray was turned off, about the same time when FLAPS1 light source was switched to HeCd, downward trending data traces that rough paralleled that of FLAPS2 could be seen. This observation suggests that the three light

sources produced fluorescent signals that reflected the dynamic presence of BG spore particles in the chamber. As for the FLAPS1 data set, what was seen here confirmed an earlier experiment shown in figure 2.

In figure 6, the FLAPS1 data trace appears disjointed due to switching between slow and regular flow rates. It gave the appearance of slightly different particle counts rates under each flow regime. In contrast, the FLAPS2 curve is a smooth trace disrupted only by time gaps reflecting changes in experimental conditions. We tried to mathematically compensate for the different flow rate by normalizing the particle counts. Slow flow values were adjusted by a factor of 1.567 to account for the flow differential. Figure 7 shows the resultant smooth curve with a characteristic similar to that for the FLAPS2 data set (figure 6).

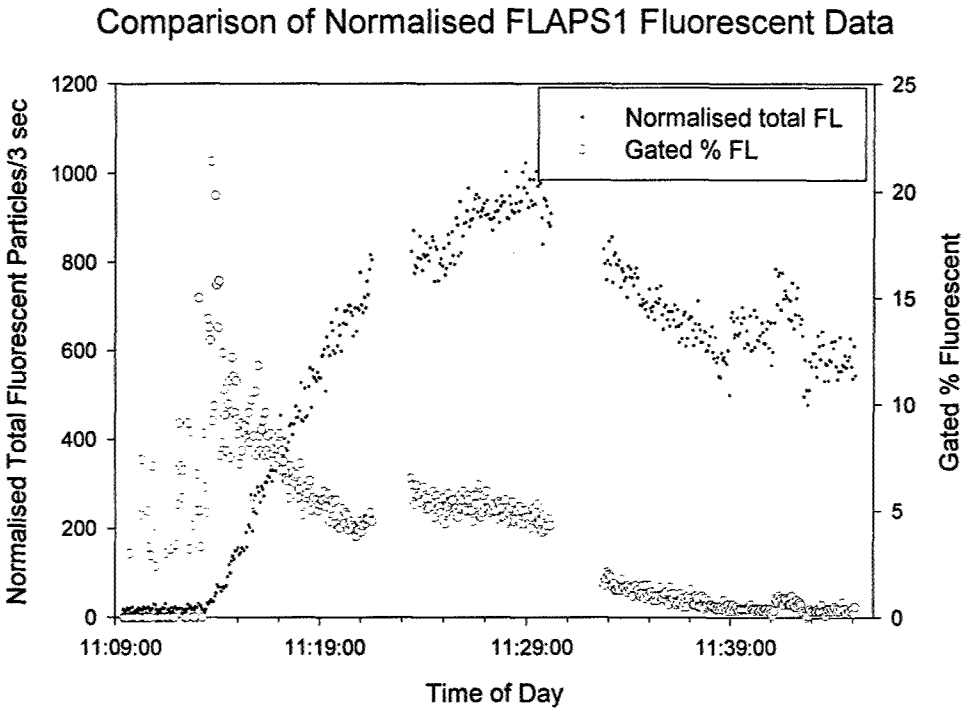

Figure 7. Replotting of FLAPS1 total fluorescence data in two ways to compensate for slow flow rates. Normalisation to the data set was done by multiplying the values obtained under slow flow conditions by 1.567. This correction factor was derived from the ratio of normal to slow flow rates. The second plot shows gated fraction of particles that fluoresce expressed as percent. The timing events are similar to those in figure 6.

Another way to present FLAPS data is to show the fraction of particles greater than a particle size cut, expressed as percentage of total particles measured. This has been shown to better reflect biological content in environmental conditions where absolute particles may fluctuate over time but the size distribution is expected to remain fairly constant if there were no unnatural disturbances. In figure 7, we also plotted the data as gated % fluorescent illustrating a different dynamic expression of the aerosol conditions in the chamber. In this example the gated fraction represents particles >2 μm as a percentage of the total particles at a given time. This is a measure of spore aggregates rather than individuals. As can be seen figure 7, before start of spray, the gated % fluorescent value was highly variable, due to low count statistics. Soon after spray on (11:12), a dramatic increase in the gated fraction could be detected. The drop in this value over extended time reflects changes in particle size dynamics. For example, the larger population may have been reduced due to falling out more rapidly with respect to the smaller particles. The restoration to regular flow at 11:24 also affected the population balance of the larger size fraction. It appeared that at 1 liter per minute, more of the larger particles were measured.

Statistical Correlation of Data Sets From Two Light Sources

We have previously shown how to compare two data sets from different measurement methodologies to determine a correlation coefficient (Ho et al 1999). By this exercise, it was demonstrated that the two data sets reflected measurements of one phenomenon. As an illustration, we plotted the fluorescent particle data sets from the two FLAPS instruments under the experimental conditions as described in figure 6. It must be pointed out that the FLAPS1 data set consisted of flow normalised values as well as data that reflect light source switching. As shown in figure 8, FLAPS data sets were not normally distributed so non-parametric statistical methods were used to derive a correlation coefficient (Spearman rank order). A value of 0.86 was obtained, suggesting a good correlation. This exercise suggests that the two instruments were measuring the same aerosol cloud in the test chamber, tracking its concentration changes with similar fidelity. Moreover, during this process, there was a light source swap in FLAPS1 suggesting that this change exerted no significant effect in the measurements.

Field Measurements of Biological Aerosol

The previous experiments done in an aerosol chamber allow controlled studies to be performed reproducibly under similar specified conditions. However, biological aerosol detection is done in the natural environment under unpredictable conditions. Thus we need to show that the FLAPS could perform comparably using the Nichia and the HeCd sources. For this exercise, the instrument was installed in a trailer and the whole device located in the field. The air intake was about 5 meters above ground level. Reference samplers (slit samplers) were positioned about 10 meters due west, 2 m above ground level. Although this set up was not optimal, the two sampling systems being spatially apart, under ideal weather conditions (west to east wind direction), distortions could be minimized (Ho et al 1999).

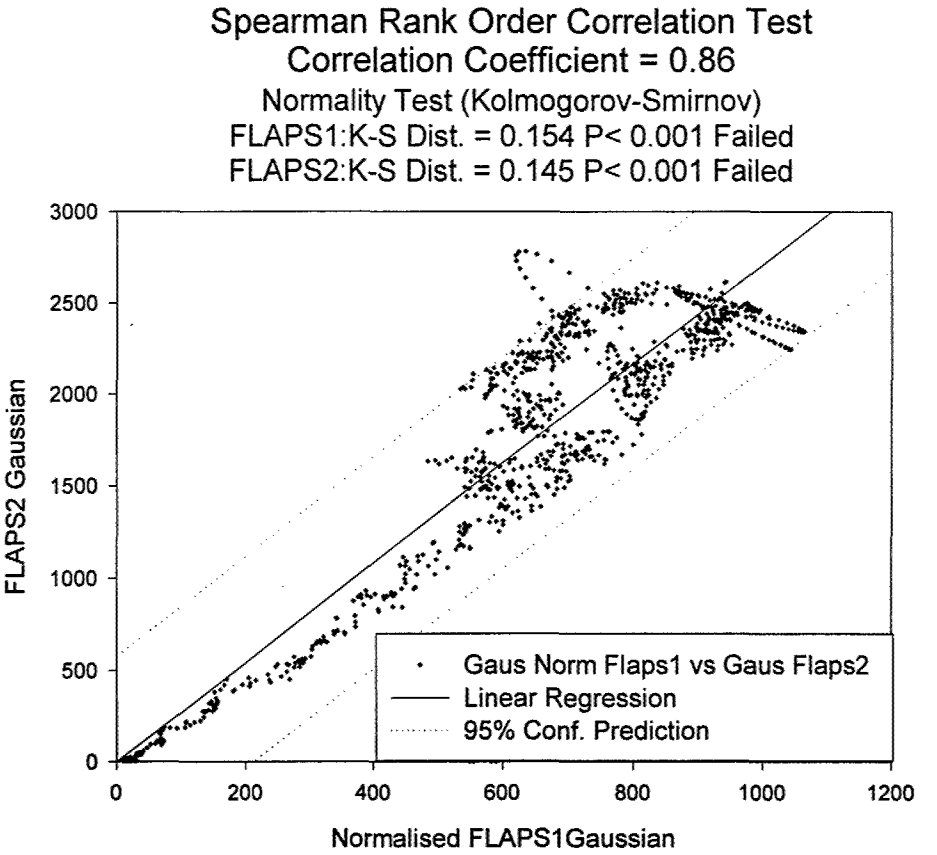

Figure 8. Statistical plot to demonstrate the two instruments (FLAPS1 and FLAPS2) were measuring the same aerosol cloud. Total fluorescent particles per 3 seconds data sets were compared after normalisation of time intervals using Gaussian estimate. A Spearman coefficient of 0.86 suggests that the two instruments responded close to synchrony when presented with a biological cloud.

The performance of the system using the Nichia light source is shown in figure 9. In the presence of a high concentration of BG aerosol, visual inspection of the fluorescence signals suggests that they coincided with that of the viable counts, expressed as agent containing particles per liter of air (ACPLA). A repeat of this, on a different day, using low aerosol concentrations (6-10 ACPLA) yielded encouraging results (figure 10). At this marginal concentration challenge level, it is gratifying to note that the instrument was performing at measurement well above the noise floor. In comparison, FLAPS with the HeCd light source gave similar measurements (figure 11).

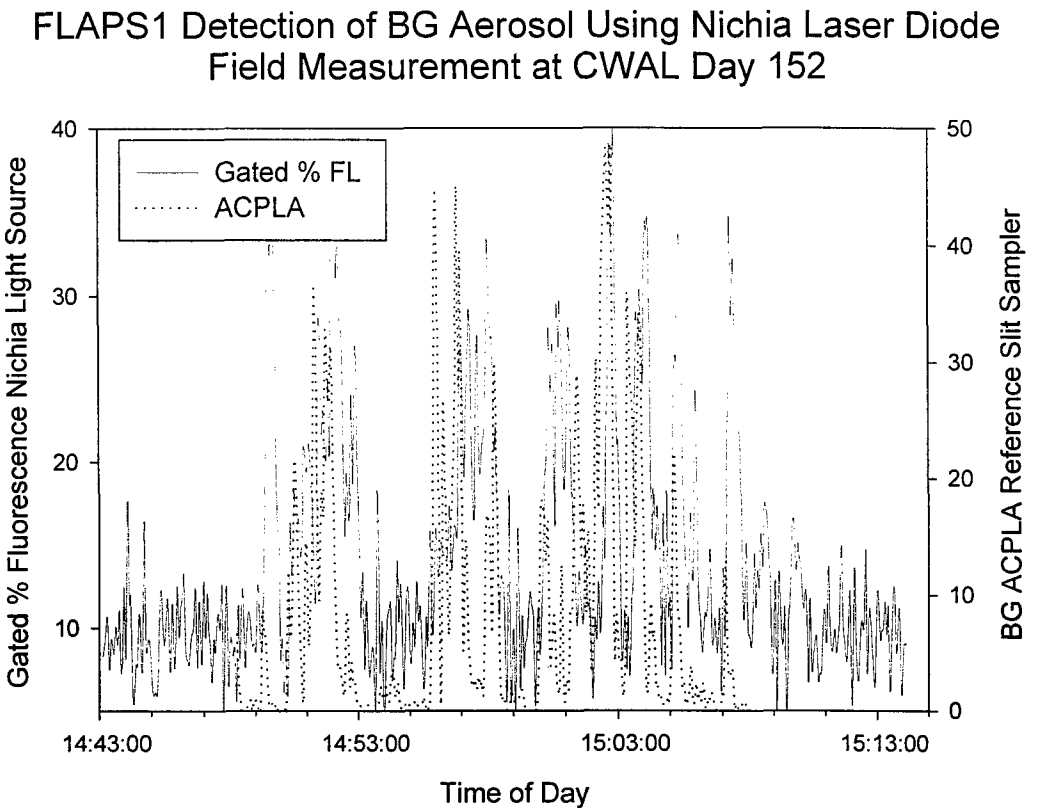

Figure 9. Field measurement of BG aerosol using Nichia light source, test 1. Out door trials were done using a Micronair sprayer to challenge the detection system.

Background Aerosol in the Field

In practice, almost all of the time, a biological detector is measuring material that occurs in the background. In the rare instance when an unusual biological cloud appears, the instrument must be capable of registering a measurement difference. Hence, the background measuring performance of an instrument must be carefully characterized.

We have emphasized the importance of using an aerosol concentrator to enhance particle counts presented to the FLAPS intake (Ho et al 1999). Figure 12

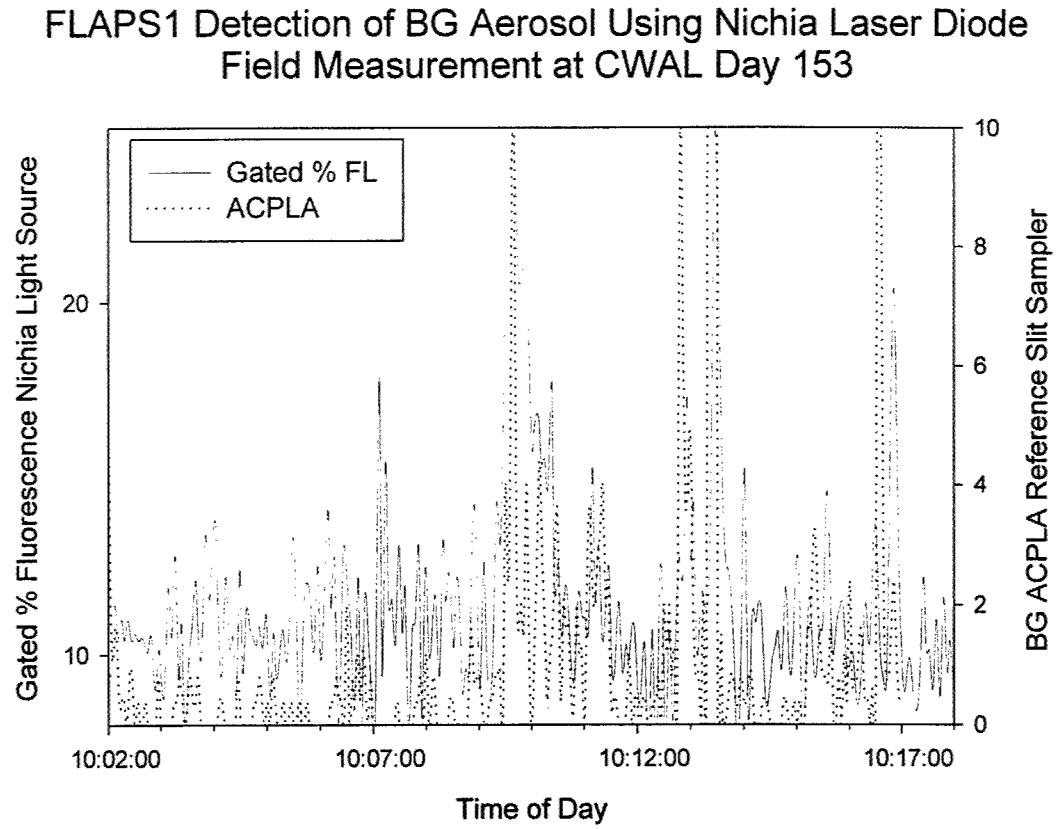

Figure 10. Field measurement of BG aerosol using Nichia light source, test 2. Since outdoor trials are associated with high variability due to environmental influences, numerous trials are required to demonstrate reproducibility. Note that even though experimental conditions used here were similar to that in figure 9, the biological aerosol concentration observed is much lower.

shows measurement of background aerosol by the Nichia equipped FLAPS with concentration with a virtual impactor. Over the 4.5 hour period, the fractional background fluorescent population showed a very gradual changes. There were the occasional spikes at different times but these were short time phenomena. Similarly, switching over to the HeCd source yielded comparable results. The slightly lower fractional levels in the latter trace may be a function of the laser power (15 vs 12 mW) although this has not be verified. Nevertheless, the two measurement systems were within acceptable performance levels, given the influence of environmental variables.

Measurement of Ovalbumin Aerosol in the Field

Ovalbumin (OV) has been used as a simulant for protein based toxins. Excitation of pure ovalbumin with UV light at 340-60 nm should not produce

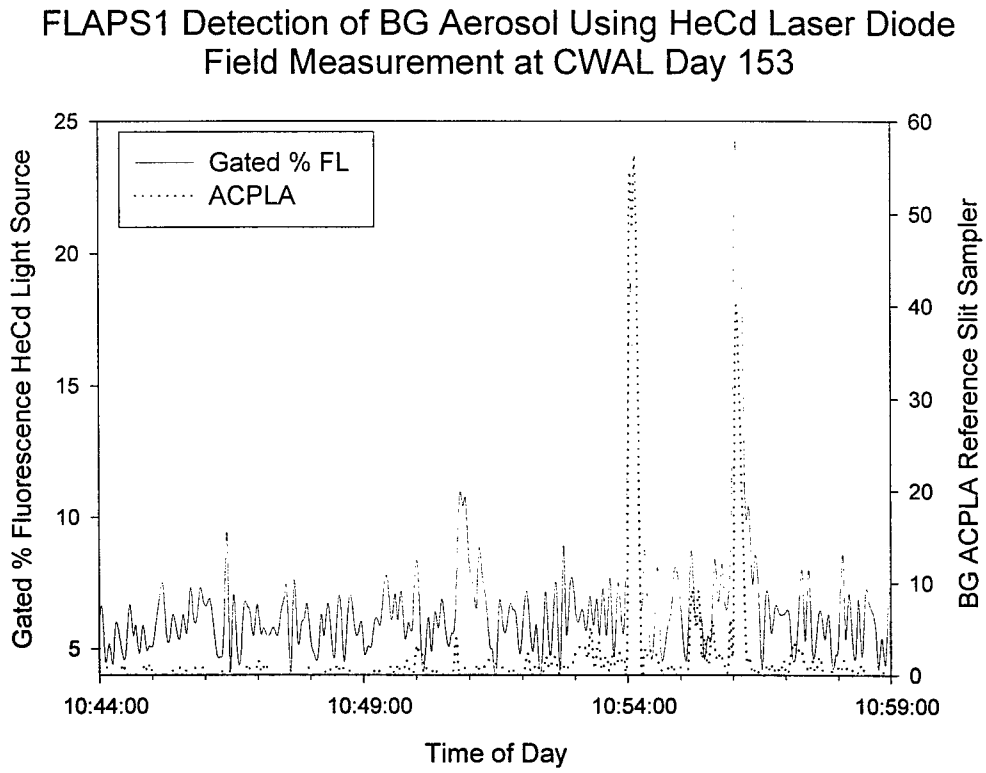

Figure 11. Field measurement of BG aerosol using HeCd light source. In this experiment, the aerosol hit the detector twice as very brief puffs.

fluorescence. Only short UV light at 260-80 nm can produce fluorescence from aromatic amino acids (Groves et al. 1968). However, in previous field trials with OV aerosol (Boulet et al. 1996), we measured fluorescence in these particles, a phenomenon we attributed to contaminants that came with commercial sources of ovalbumin. Indeed, the Sigma Chemical Co. material that was used for the current experiments, on visual inspection, appeared to have a yellowish tinge, suggesting the presence of egg yolk contaminants. As shown in figure 13, OV aerosol could be detected when using the Nichia light source, very comparable to that done with the HeCd source (figure 14). In these examples, we also illustrated the performance of the alarming algorithm in the presence of OV above background aerosol. The higher

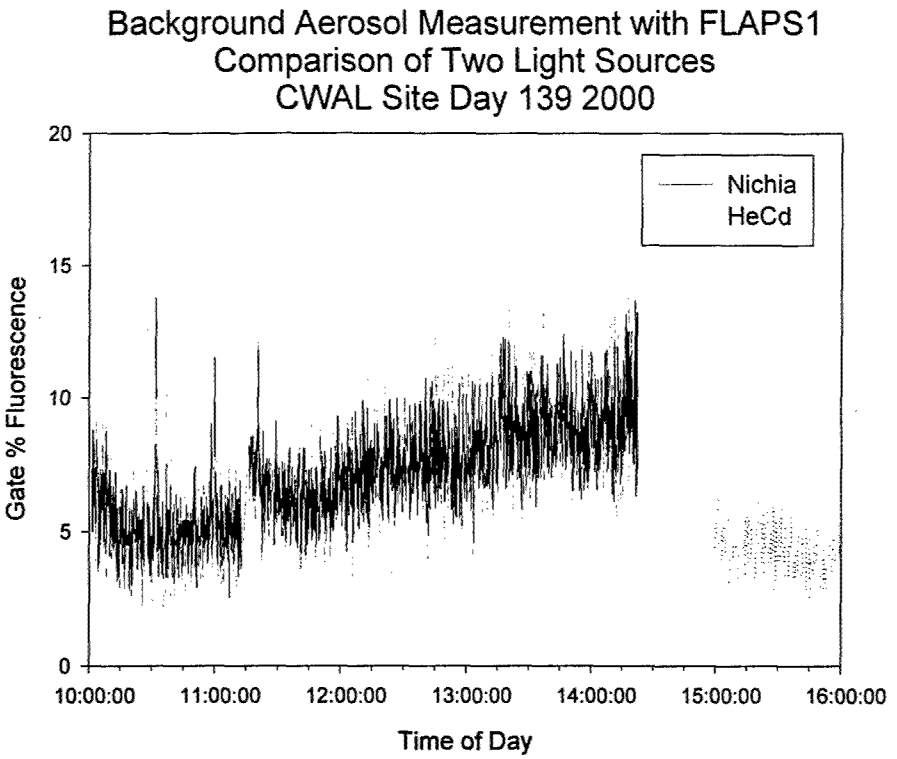

Figure 12. Comparison of background aerosol measurement using the Nichia and HeCd light sources. Power settings were 15 mW and 12 mW respectively. Due to slight differences in operational characteristics between the two sources, the gated % fluorescence level for Nichia always registered higher than that for the HeCd source. But the difference was not great enough to cause problems with interpretation of data in the presence of biological aerosol clouds as seen in the following figures.

background noise level in figure 13 vs 14 could be due to environmental conditions for that day. This was typical for what has been observed in different locations in different parts of the world. Despite that, the alarming software response appeared to be unaffected.

We regret to note that reference data for OV was not available for comparison. Current methods for collecting OV aerosol is by glass impinger and the time resolution for this device is 5 minutes. Data from this collection method would have been unsuitable for comparison with FLAPS data sets; the latter system has a time resolution of 4 seconds.

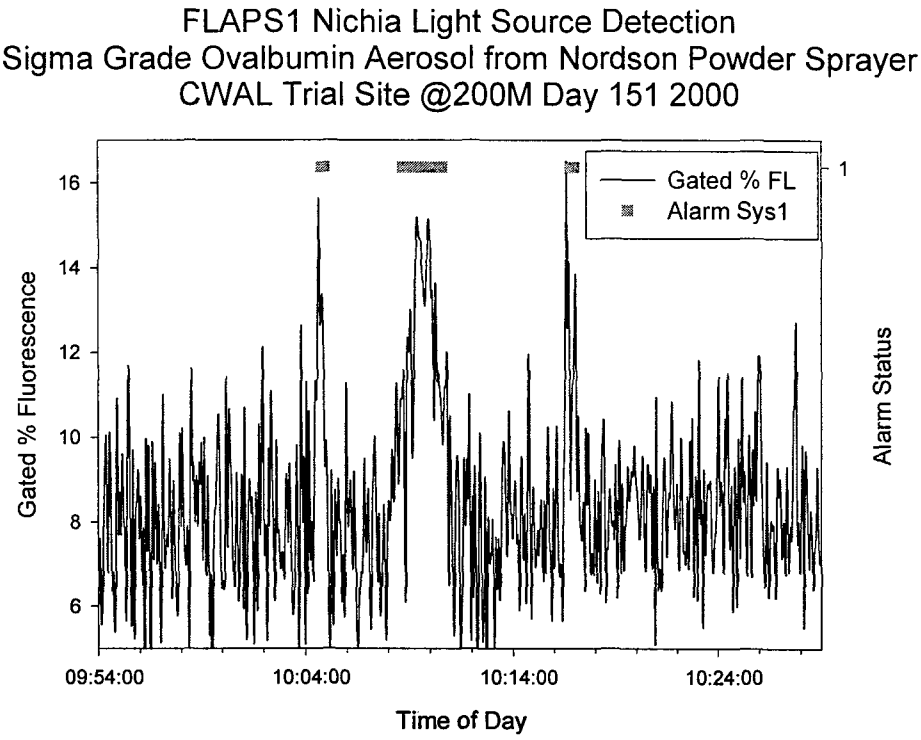

Figure 13. Detection of crude Sigma grade ovalbumin with Nichia light source in FLAPS1. A Nordson powder sprayer was loaded with ovalbumin in 15% Syloid added to enhance flow characteristics. The sprayer was located 200 m upwind of the detector.

Biomolecules Excited by 402 nm Light

In the original FLAPS design where 340-60 nm excitation light was used, we speculated that the fluorescing molecules were most probably NADH (figure 15). With the Nichia light source emitting at 402 nm, it is possible that the excited molecule is riboflavin as illustrated in the figure taken from Li et al (1991). This illustration also suggests that biological molecules have broadband excitation and emission spectral characteristics. Hence, it can be seen that at this 402 nm wavelength, a small portion at the upper end of NADH excitation spectrum may have a small contribute to the total fluorescing signals. To our knowledge, measurement of riboflavin in spores has not been reported so it is impossible to verify this claim. However, in the literature, autofluorescence from blue and near UV excitation has been reported for a brackish water ciliate that feeds on cyanobacteria (Selbach and Kuhlmann, 1999). Also Van Schaik et al (1999).

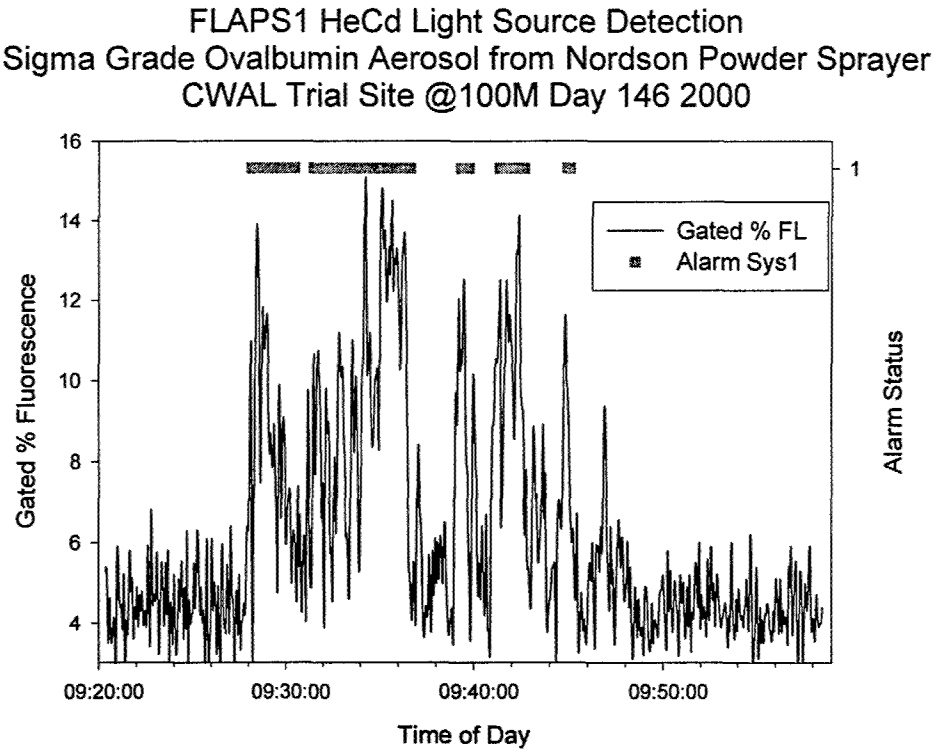

Figure 14. Detection of crude Sigma grade ovabumin with native HeCd light source in FLAPS1. A Nordson powder sprayer was loaded with ovalbumin in 15% Syloid added to enhance flow characteristics. The sprayer was located 100 m upwind of the detector.

attributed 405 nm excited fluorescence in cornea of diabetes mellitus patients to flavins. Finally Solovieva et al (1999) reported of isolation of the *Bacillus subtilis* DNA that contained the riboflavin operon and was able to clone it to *E. coli*. At least this suggests that BG has the capability to synthesize riboflavin. Whether the individual spore contains significant amounts of the material is difficult to predict.

Significance of Using a Laser Diode as Light Source.

Current commercially purchased 340-360 nm laser devices rely on frequency tripling techniques to down convert light from a 1060 nm laser diode. Although the starting light source is reliable, the frequency tripling optics requires many components that work at very narrow environmental conditions. This "smoke and mirror" approach works well in the laboratory but poses disastrous consequences in

field applications where operational conditions are extreme. Current solutions involved expensive air conditioning and shock mounting, making the final detector system expensive to manufacture and operate. The implication from this lesson is that a complete departure from frequency tripling technologies is the only viable route to pursue. For this reason, we have elected to find a light source that does not suffer from these handicaps. Weighing 0.3 gm, the Nichia laser diode meets the physical characteristics and in this paper we have shown that it also meets biological requirements.

Another major consideration is cost; in 2000-01, a conventional frequency tripled device costs about US$30000, while a single Nichia laser diode can be obtained for US$2K. With volume production, Nichia may lower this even further, in line with red diodes that typically cost less than $100. The closest alternative to our solution is the "DualChip NanoUV 355" (JDSUniphase, Horsham, PA), a passively Q-switched laser that produces light at 355 nm. It outputs average power at 50 mW by utilizing harmonic generation stages integrated in the laser head. But due to its reliance on "smoke and mirror" technologies, operating temperature is 15-35° C. Without extra temperature control systems, it weighs 9 kg and costs about US$10K. At this level, any future biological detector still based on frequency tripled technologies will face severe competitive pressures in the military as well as civilian market place, reliability factor notwithstanding.

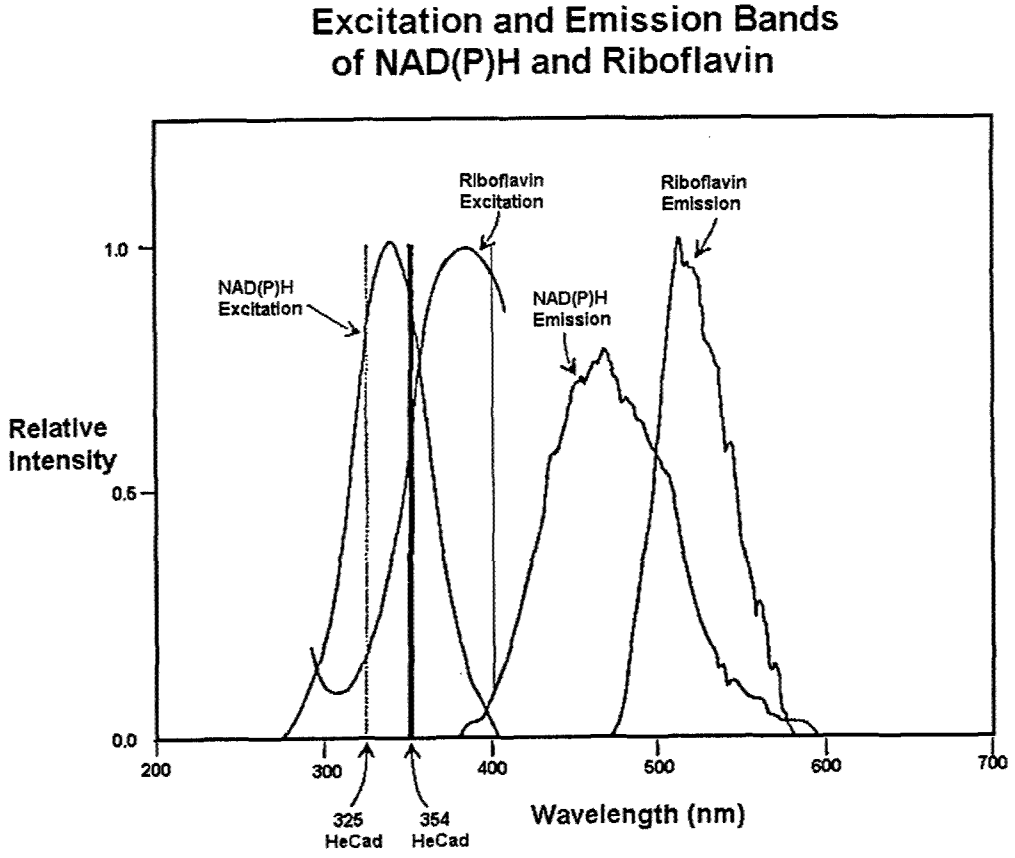

Figure 15. Excitation and emission spectra of different biomolecules associated with cellular metabolism.

CONCLUSIONS

We have demonstrated that FLAPS performance using the two light sources gave very similar characteristics when presented with a range of biological aerosol simulants. Most encouraging was the similar fluorescence response from both excitation sources in the presence of spore aerosol; a material considered the most difficult to detect optically. Spores, by their physiological nature, contain very little biological material and the individual units are no larger than 0.7 μm and enclosed by a very refractile and chemical resistant spore coat. Due to these characteristics, conventional light microscopy of spores reveal very little intercellular content. In addition, dyes in general do not penetrate the spore coat, making flow cytometric studies difficult. In contrast, vegetative cells are better subjects for examination in that they can easily be stained. Thus, performance of biological detectors must be rated by their ability to measure spore aerosols and in these experiments, it has been shown that 10 ACPLA could be detected (figure 10).

In summary, we have shown that by using a small laser diode that emits at 402 nm, it is possible to replicate the performance of 340-60 nm light sources. The findings from this study have critical implications for the future of biological detection, both for military applications as well as in environmental monitoring. With this new light source, a detector will require no active cooling system, consume little power and weight much less than current instruments. The associated cost reduction will not only benefit defence applications but will make it possible for industrial, or home use.

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In this paper, we report on our continuing attempts to improve on the FLAPS technology. The frequency tripled laser light source in FLAPS2 has been replaced by a small laser diode, with the potential to reduce cost by several orders of magnitude. The main focus of this paper will be to demonstrate that the instrument with a new light source performs similarly to previous generations of FLAPS.

Laboratory and field trial data indicate that FLAPS performance using the two light sources gave very similar performance characteristics when presented with different biological aerosol simulants. Most encouraging was the similar fluorescence response from both excitation sources in the presence of spore aerosol; a material considered the most difficult to detect optically. Spores, by their physiological nature, contain very little biological material and the individual units are no larger than 0.7 μm and enclosed by a very refractile spore coat. Due to this refractile characteristic, conventional light microscopy of spores reveal very little of its cellular content. In contrast, vegetative cells are better subjects for examination in that they can easily be stained. Thus, performance of biological detectors must be rated by their ability to measure spore aerosols and in these experiments, it has been shown that 10 ACPLA could be detected.

In the original FLAPS design where 340-60 nm excitation light was used, we speculated that the fluorescing molecules were most probably NADH. With the Nichia light source emitting at 402 nm, it is possible that the excited molecule is riboflavin as illustrated in the figure taken from Li et al (1991). In addition, it can be seen that at this wavelength, a small portion at the upper end of NADH excitation spectrum may have a small contribute to the total fluorescing signals. To our knowledge, measurement of riboflavin in spores has not been reported so it is impossible to verify this claim. However, in the literature, autofluorescence from blue and near UV excitation has been reported for a brackish water ciliate that feeds on cyanobacteria (Selbach and Kuhlmann, 1999). Also Van Schaik et al. (1999) attributed 405 nm excited fluorescence in cornea of diabetes mellitus patients to flavins. Finally Solovieva et al (1999) reported of isolation of the *Bacillus subtilis* DNA that contained the riboflavin operon and was able to clone in to *E. coli*. At least this suggests that BG has the capability to synthesize riboflavin. Whether the individual spore contains significant amounts of the material is difficult to predict.

In summary, we have shown that by using a small laser diode that emits at 402 nm, it is possible to replicate the performance of 340-60 nm light sources. The findings from this study have critical implications for the future of biological detection, both for military applications as well as in environmental monitoring. With this new light source, a detector will require no active cooling system, consume little power and weight much less than current instruments. The associated cost reduction will not only benefit defence applications but will make it possible for industrial or home use.

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